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Synergistic Activity of Trimethoprim and Amikacin Against Gram-Negative Bacilli

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The in vitro effect of trimethoprim on the inhibitory and bactericidal activity of amikacin against 20 strains each of *Klebsiella pneumoniae* and *Serratia marcescens*, 15 strains of *Escherichia coli*, and 10 strains of *Pseudomonas aeruginosa* was examined by the checkerboard technique in microtiter plates. Trimethoprim had a synergistic effect on the inhibitory and bactericidal activity of amikacin against the majority of non-pseudomonas strains tested. The mean ± standard deviation fractional inhibitory concentration indexes were 0.59 ± 0.19 for the *Klebsiella* strains, 0.48 ± 0.18 for the *Serratia* strains, and 0.60 ± 0.22 for the *E. coli* strains tested. Respective mean ± standard deviation fractional bactericidal concentration indexes for these organisms were 0.55 ± 0.17, 0.54 ± 0.29, and 0.61 ± 0.22. A total of 40% of the *Klebsiella* strains, 80% of the *Serratia* strains, and 46% of the *E. coli* strains had a fractional inhibitory concentration equal to or less than 0.25 for both of these antimicrobial agents and were considered to be synergistically inhibited by the combination. By applying this criterion to bactericidal activity, synergy was demonstrated against 50, 65, and 46% of these strains, respectively. All of the *Enterobacteriaceae* tested were inhibited by clinically achievable concentrations of trimethoprim and amikacin. Antagonism was not demonstrated with any of the organisms tested. Trimethoprim had no antibacterial effect on the *Pseudomonas* strains and did not alter amikacin's activity against these bacteria.

Trimethoprim in combination with sulfamethoxazole is a well-studied example of antimicrobial synergy with activity against a wide variety of bacteria (1). Previous studies have demonstrated synergy with the trimethoprim-sulfamethoxazole combination and polymixins against gram-negative bacteria (3, 6, 9). Also, trimethoprim and colistin have shown synergy against strains of *Enterobacteriaceae* and *Pseudomonas aeruginosa* (3, 6, 8). This report examines the effect of trimethoprim on the in vitro activity of amikacin against strains of *Klebsiella pneumoniae, Serratia marcescens, Escherichia coli,* and *P. aeruginosa*.

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MATERIALS AND METHODS

Minimal inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC) were determined for amikacin in the presence of increased concentrations of trimethoprim. A total of 20 strains of *K. pneumoniae*, 20 strains of *S. marcescens*, 15 strains of *E. coli*, and 10 strains of *P. aeruginosa* were studied. All organisms were isolated from hospitalized patients.

Organisms that had been stored on corked agar slants were transferred to blood agar plates and then grown overnight in Mueller-Hinton broth. Stock solutions of trimethoprim and amikacin were prepared in concentrations of 1,000 μg/ml by dissolving the antimicrobial powders in Mueller-Hinton broth. Trimethoprim base powder required the addition of 0.1 N lactic acid to achieve solubility (1). Stock solutions were stored frozen in multiple vials, allowing a single volume to be thawed for each day's testing.

All subsequent organism and antimicrobial dilutions were made with Mueller-Hinton broth plus 5% lysed horse blood (obtained by alternate freezing and thawing of defibrinated horse blood).

Combined activity of the two drugs was evaluated by a modification of the checkerboard technique in microtiter plates, as described previously by Zinner et al. (10). The final volume in each microtiter well was 150 μl, consisting of 25 μl of each antimicrobial solution, 25 μl of the bacterial suspension diluted to a final concentration of 10^6 organisms per ml, and 75 μl of diluent broth. To those wells receiving only one antimicrobial solution an additional 25 μl of diluent was added, and 50 μl of diluent was added to the

antibiotic-free control well. A sterile 4-mm magnetic stirring bar was added to each microtiter well. Before incubation and subsequent sampling, the microtiter plate was placed on a magnetic stirrer to ensure adequate mixing of the well contents.

The microtiter plates were then incubated overnight at 37°C. After incubation, the MIC for each antimicrobial alone and in combination was obtained by inspecting the wells for turbidity. A replicator type inoculator, constructed to conform to the microtiter wells, was used to sample approximately 2 μl from each well and inoculate it onto duplicate antibiotic-free Trypticase soy agar plates. These agar plates were then incubated overnight at 37°C. The MBC for each antimicrobial agent alone and in combination was then obtained by inspecting the inoculation sites for evidence of growth. Two colonies or less at the site represented 99.9% reduction of the original inoculum.

The rate of antimicrobial killing alone and in combination was assessed for selected organisms by a standard pour-plate technique.

To compare the antibacterial effects of the trimethoprim-amikacin combination on organisms of varying susceptibilities, the data for each organism were expressed as fractional inhibitory concentrations (FIC) and FIC indexes. As defined by Elion et al. (2), the FIC is the ratio of the MIC of a drug in combination to the MIC of the drug alone expressed as a decimal fraction. The FIC index is the sum of the FICs for each of the drugs in a particular combination. When the FIC index is less than unity, synergy is suggested; relatively lower index values indicate a relatively greater degree of antimicrobial synergy. An FIC index less than 1 also may be depicted graphically by an isobologram in which there is a downward bowing of the actual isobol, away from the diagonal, theoretical additive isobol (7). The MBC data are expressed similarly by fractional bactericidal concentrations (FBC) and FBC indexes.

RESULTS

The FIC index values for each of the *Klebsiella, Serratia,* and *E. coli* strains tested are shown in Fig. 1. The majority of these strains had FIC indexes equal to or less than 0.75. A similar distribution was found for the fractional bactericidal indexes (not shown). The mean FIC and FBC index values for these organisms are shown in Table 1. All of the *Enterobacteriaceae* strains were inhibited by 1.6 μg or less of trimethoprim alone per ml and by 6.4 μg or less of amikacin alone per ml.

Considering the twofold (or one dilution) variations associated with the serial dilution method, each organism was also assessed individually for evidence of synergistic inhibition. For this analysis, the antimicrobial combination was considered synergistic only when the MIC or MBC for each drug in combination was at least fourfold lower than that of the drug alone. This is equivalent to an FIC (or FBC) of 0.25 or less for each drug and, thus, an FIC (or FBC) index value of 0.50 or less. These criteria are expressed in isobologram form in Fig. 2. The results obtained by this definition of synergy are presented in Table 2. Overall, trimethoprim and amikacin were definitely synergistic against over 50% of the organisms studied. Organisms not fitting these criteria were affected in at least an additive fashion. Antagonism was not seen in any of the strains tested.

TABLE 1. *Mean FIC and FBC indexes for trimethoprim and amikacin in combination*

Strain	Mean FIC index[a]	Mean FBC index[a]
K. pneumoniae	0.59 ± 0.19	0.55 ± 0.17
S. marcescens	0.48 ± 0.18	0.54 ± 0.29
E. coli	0.60 ± 0.22	0.61 ± 0.22

[a] Mean index ± standard deviation.

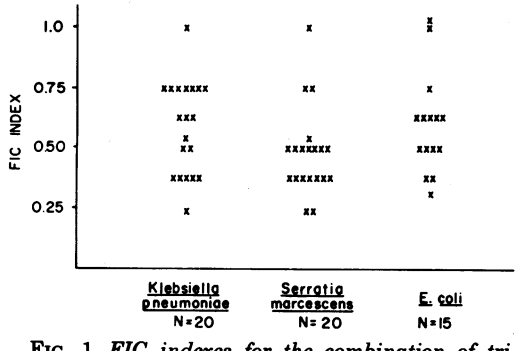

FIG. 1. *FIC indexes for the combination of trimethoprim and amikacin against individual strains (x).*

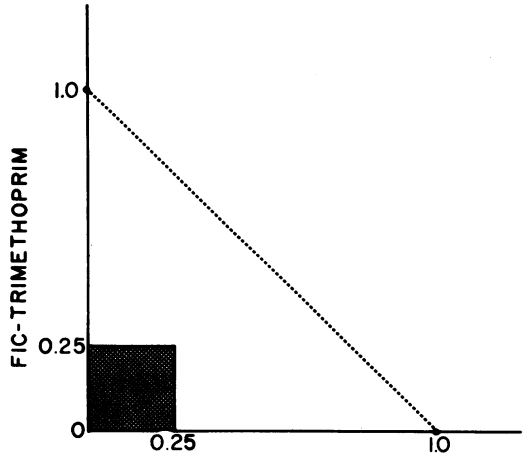

FIG. 2. *Area of defined synergy (shaded area) related to the theoretical additive isobol.*

A representative antimicrobial killing curve is presented in Fig. 3. Synergy is suggested by the observation that the trimethoprim-amikacin combination resulted in a 10^4 or greater decrease in the number of surviving organisms when compared with the activity of either drug acting alone. These results agreed with the evidence of synergy demonstrated by the microtiter method.

The 10 strains of *P. aeruginosa* tested were all inhibited by less than 5 μg of amikacin per ml. None of these strains was inhibited by trimethoprim in concentrations up to 12 μg/ml. Trimethoprim did not affect the activity of amikacin against *P. aeruginosa*.

TABLE 2. *Percentage of strains affected synergistically by amikacin and trimethoprim*

Strain	Strains (%) affected synergistically	
	MIC	MBC
K. pneumoniae	40 (8/20)[a]	50 (10/20)[a]
S. marcescens	80 (16/20)	65 (13/20)
E. coli	46 (7/15)	46 (7/15)

[a] Numbers in parentheses indicate number affected per total number.

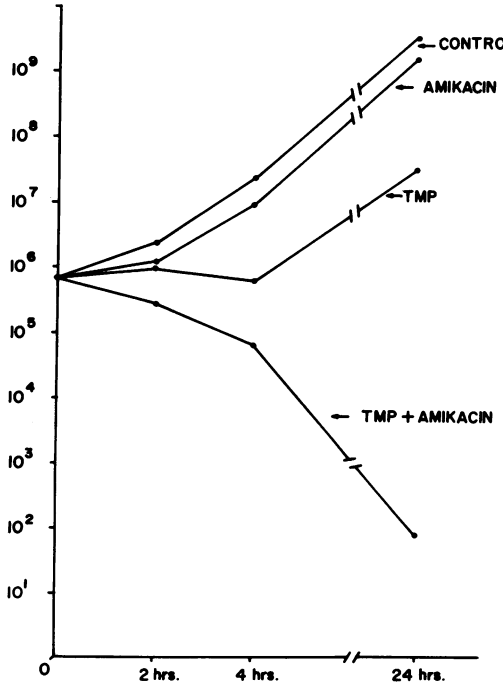

FIG. 3. *Comparative killing rates of a strain of S. marcescens by trimethoprim (0.4 μg/ml) and amikacin (0.4 μg/ml) alone and in combination. Units on the ordinate express number of organisms per milliliter.*

DISCUSSION

These data suggest that trimethoprim and amikacin may act synergistically in vitro against selected gram-negative bacilli. The mean FIC indexes for the strains of *K. pneumoniae* (0.59), *S. marcescens* (0.48), and *E. coli* (0.60) tested were all less than 1.0 and are consistent with a synergistic effect. A total of 40% of the *Klebsiella* strains, 80% of the *Serratia* strains, and 46% of the *E. coli* strains satisfied a more stringent definition of synergistic effect, designed to account for the variability inherent in the serial dilution method. Similar data were obtained with an estimate of bactericidal activity of these drugs in combination.

The lack of synergistic effect seen with *P. aeruginosa* strains is consistent with the previously observed resistance of this organism to trimethoprim (1). This resistance is felt to be mediated by the impermeability of the *Pseudomonas* cell wall to trimethoprim (1). The presence of amikacin did not enhance the activity of trimethoprim against these *Pseudomonas* strains.

The mechanism for the apparent synergy against the *Enterobacteriaceae* tested is unexplained. Synergy was demonstrated for these organisms with concentrations of amikacin and trimethoprim that are clinically achievable (4, 5).

The clinical significance of the demonstrated in vitro synergy remains to be determined. The possibility for concurrent clinical use of these two antimicrobial agents certainly does exist, especially in immunosuppressed patients. The potential value of combination chemotherapy is also a consideration in the treatment of multiply drug-resistant infections (9). However, it should be stressed that all strains of *Enterobacteriaceae* tested were sensitive to both amikacin and trimethoprim. Further studies of multiply drug-resistant bacteria and the effect of trimethoprim on other aminoglycosides are indicated.

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